Jet Propulsion Laboratory
California Institute of Technology

The Importance of Lyophilization in Space Research

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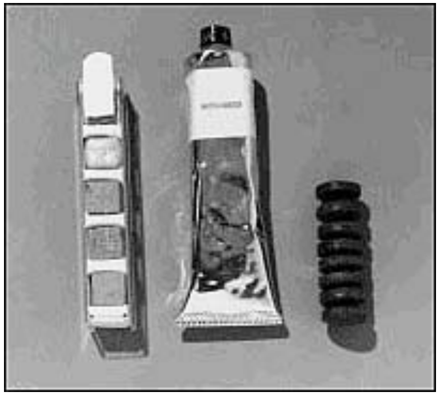
2018 FREEZE DRYING OF PHARMACEUTICALS AND BIOLOGICALS
Short Course and Conference
Sept 18-21, 2018
Garmisch-Partenkirchen, Germany

Background

- The complete removal of water from a target while leaving the structure and composition intact is an essential tool that is leveraged extensively in the space industry.
- Freeze Drying is advantageous to solve several problems:
  1. Preservation of products with a limited shelf life
  2. Transportation and storage ease
  3. Relevant simulation conditions for space research

- The spoilage of food stems from decomposition via microorganisms as well as naturally occurring enzymes in the food that react with oxygen to result in ripening and spoiling.
- Food selection in the past
  - NASA's early astronauts survived the age of bite-sized snack cubes, freeze-dried powders, and semi-liquids dispensed in aluminum tubes.
  - The consensus was that the foods were unappetizing, difficult to rehydrate, and the crumbs posed a hazard to the instrumentation.
  - The situation improved for the Gemini missions: Improved packaging gave way to improved food quality and made reconstitution easier.
  - Apollo astronauts were first to have hot water, which greatly enhanced the rehydrating process and the taste of the food.
- Food selection today
  - Astronauts now have a variety of food choices available which rely heavily on the lyophilization process as well as thermostabilization.



Early Project Mercury food tube and dry bite-sized snacks with gelatin coating. Source: NASA.gov

Space food selection today, which includes freeze dried and thermostabilized options. Source: NASA.gov

1. Preservation of products with a limited shelf life – Quantitative Life detection methodologies

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Frequently-used Planetary Protection quantitative analytical methods for microbial detection leverages lyophilization to produce shelf-stable reagents/primers.

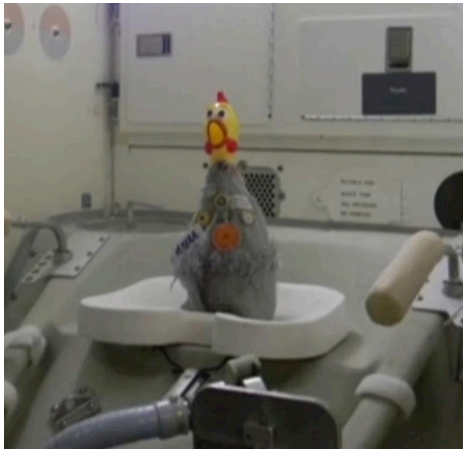
Assay	Target	Microbial kinds	Instrument	Reagent
NASA heat-shock (80˚C; 15 min); 3 days	Spores	Mesophilic; 32˚C; aerobic; heterotrophs	Cultivation	TSA
NASA non heat-shock; 7 days	Spores and vegetative cells	Mesophilic; 25˚C; aerobic; heterotrophs	Cultivation	TSA
ATP	ATP molecule	Bacteria, archaea, and fungi	Photomultiplier	Luciferin-luciferase kit
LAL	LPS, glucan	Gram negative and positive bacteria	Spectro photometer	Endochrome-K LAL reagent Kit
Q-PCR	DNA	Bacteria, archaea, and fungi	PCR machine	PCR reagent/ primers for 16S or 18S targets
Microscopy	All biological particles	Bacteria, archaea, and fungi	Sophisticated to fluorescent microscope	Dyes that could stain DNA and other molecules
FACS	All cells	Bacteria, archaea, and fungi	Flow cytometer with lasers	Dyes that could stain cells

Requires lyophilization to produce shelf-stable reagents/primers

2. Transportation and Storage Ease

- The freeze-drying process significantly reduces the total weight of the target which allows for greater transportation ease, as well as storage ease.
  - Food applications - Many food products are comprised mostly of water. Freeze drying significantly reduces the mass and ultimately the cost of transportation into space.
    - Lyophilization of food products also aides in easier storage, especially on such close-quarter spaces as the ISS.
  - Waste applications –Extended manned space missions require waste materials storage generated throughout the mission [1-3]. Lyophilization is a solution that allows for compact long-term storage

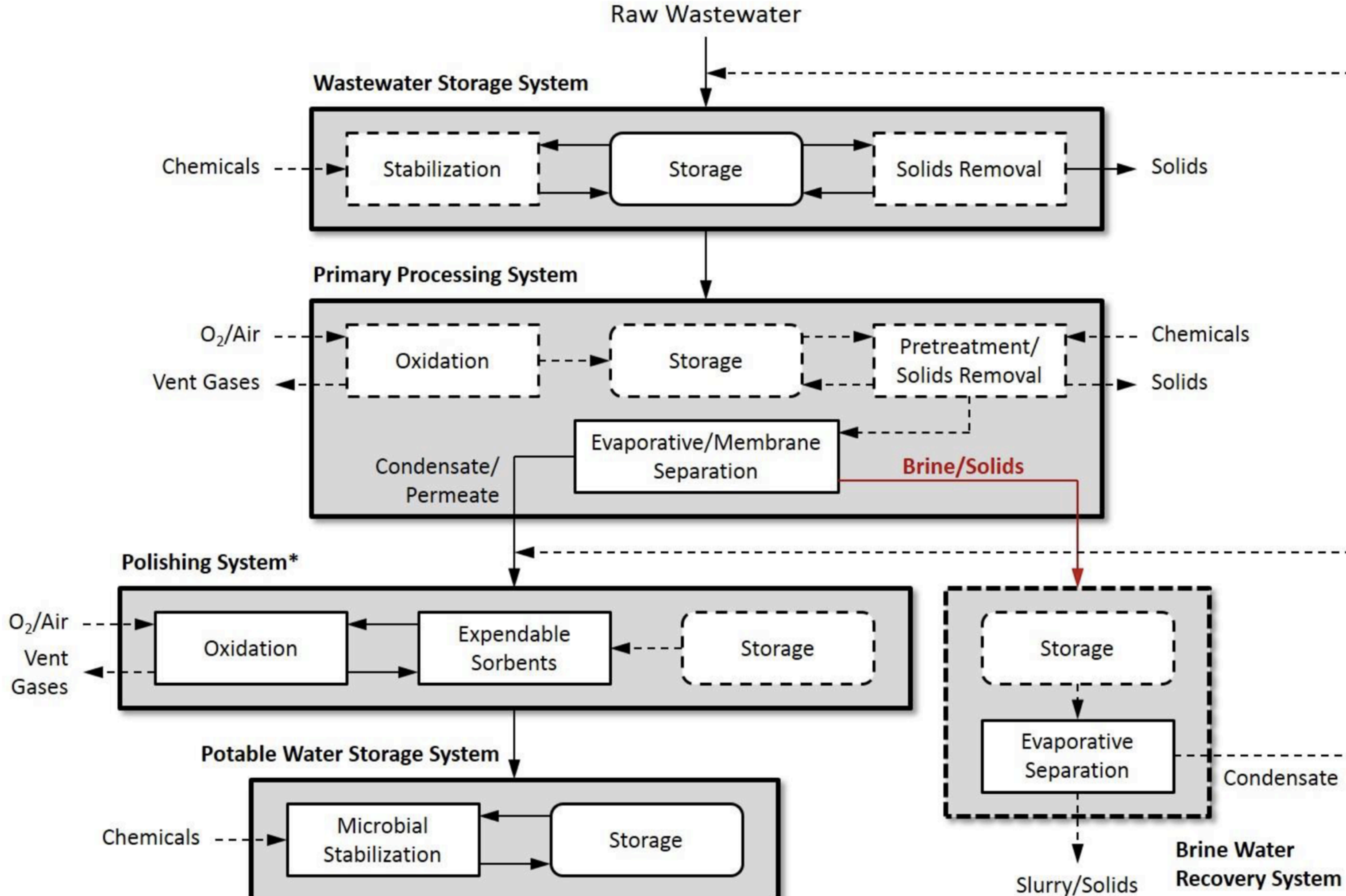
Apollo flight food packages. Source: NASA



Space waste, to include human products, must be managed effectively for extended manned missions. Source: NASA

[1] Wheeler, R., Hadley, N., Dahl, R., Williams, T. et al., "Microwave Enhanced Freeze Drying of Solid Waste," SAE Technical Paper 2007-01-3266, 2007, https://doi.org/10.4271/2007-01-3266.
[2] Parametric Analysis of Life Support Systems for Future Space Exploration Missions Michael Swickrath, Molly Anderson, Robert Bagdigian. 41st International Conference on Environmental Systems Portland, Oregon
[3] Litwiller, Eric, et al. “Lyophilization -Solid Waste Treatment.” 34rd International Conference on Environmental Systems; 19-22 Jul. 2004; Colorado Springs, CO; United States

2. Transportation and Storage Ease – Waste Products generated during extended manned space missions



"Generalized schematic of water recovery system architecture elements. Elements depicted by dotted lines may be considered optional."

Source: W. Jackson, et al. "Water Recovery from Brines to Further Close the Water Recovery Loop in Human Spaceflight." 44th International Conference on Environmental Systems,13-17 July 2014, Tucson, Arizona.

3. Relevant simulation conditions for space research - Eli Lilly-Lyophilization

- PI: Jeremy Hinds, Eli Lilly and Company, Indianapolis, IN, United States
- Co-I: Evan Hetrick, Ph.D., Eli Lilly and Company, Indianapolis, IN, United States
- Developers - NASA Glenn Research Center, Cleveland, OH, United States and ZIN Technologies Incorporated, Cleveland, OH, United States
- ISS Expedition Duration - April 2017 - August 2018
- Science Objectives: Lyophilization in Microgravity (Eli Lilly-Lyophilization) examines freeze-drying processes in the microgravity environment aboard the International Space Station (ISS). Freeze-drying may create layering or other textures in the presences of gravity. Eli Lilly-Lyophilization freeze-dries a range of samples under microgravity conditions aboard the ISS and then returns the samples to Earth for comparison with control samples.
- Results from this study will improve understanding of how food, drugs and other compounds are preserved in space, which can inform strategies for long-term space travel.

NASA Image: ISS052E075804 - Astronaut Jack Fischer works in the Microgravity Science Glovebox (MSG) work volume during Eli Lilly-Lyophilization hardware setup.

Source: https://www.nasa.gov/mission_pages/station/research/experiments/2274.html#overview

3. Relevant simulation conditions for space research - Eli Lilly-Lyophilization Research Overview

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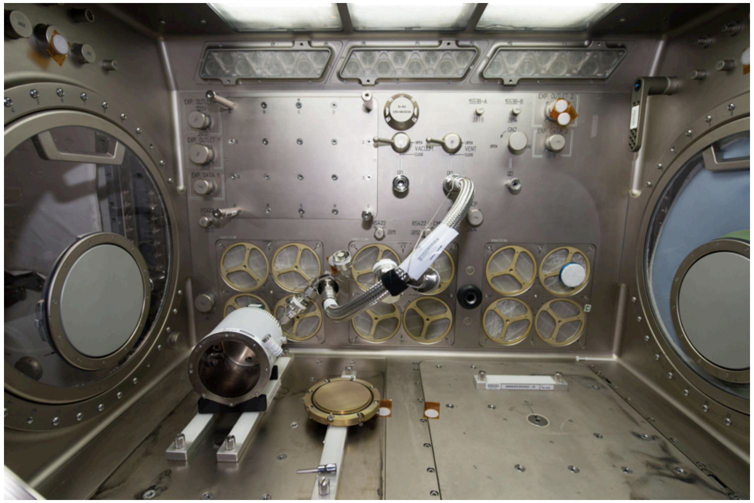
- This experiment aims to answer to the following questions:
  - Is the stratification the result of the freezing, or the dehydration process?
  - How is the stratification related to the resulting crystal form and particle size?
  - If there is no gravity, is there any stratification?
  - If there is not stratification, are there differences in resulting crystal form and particle size? If no, then which form is favored?
- The study answers the above questions by investigating three sample groups:
  - frozen prior to launch and continuous storage within ISS freezers
  - Launched with no temperature-conditioned stowage during ascent, but frozen on orbit
  - Launched with no temperature-conditioned stowage during ascent, frozen on orbit, and lyophilized in space.
- All 3 groups are returned to Earth frozen and submitted to the project scientists for additional testing.



NASA Image: ISS052E075807 (top) and ISS052E075812 (bottom) - View of Eli Lilly-Lyophilization hardware setup in the MSG work volume.

Source: https://www.nasa.gov/mission_pages/station/research/experiments/2274.html#overview

LYOPHILIZATION APPLICATIONS WITHIN THE MARS 2020 MISSION

Planetary Protection – First, let's talk about microbes!

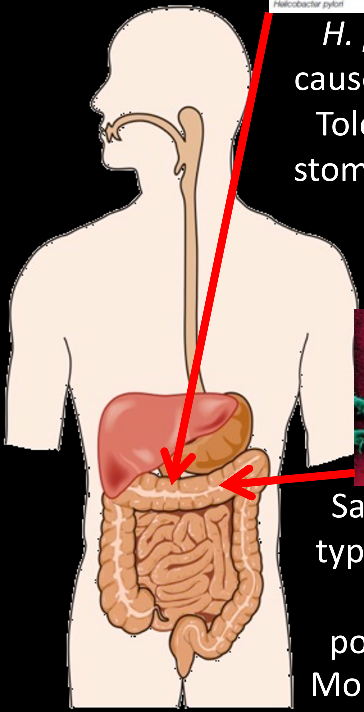
Oldest Form of Life

Microbes isolated from deserts throughout the world.

Rio Tinto, Spain: a low pH, high heavy metal environment.

Live in harsh environments

H. pylori – causes ulcers. Tolerant to stomach acid.

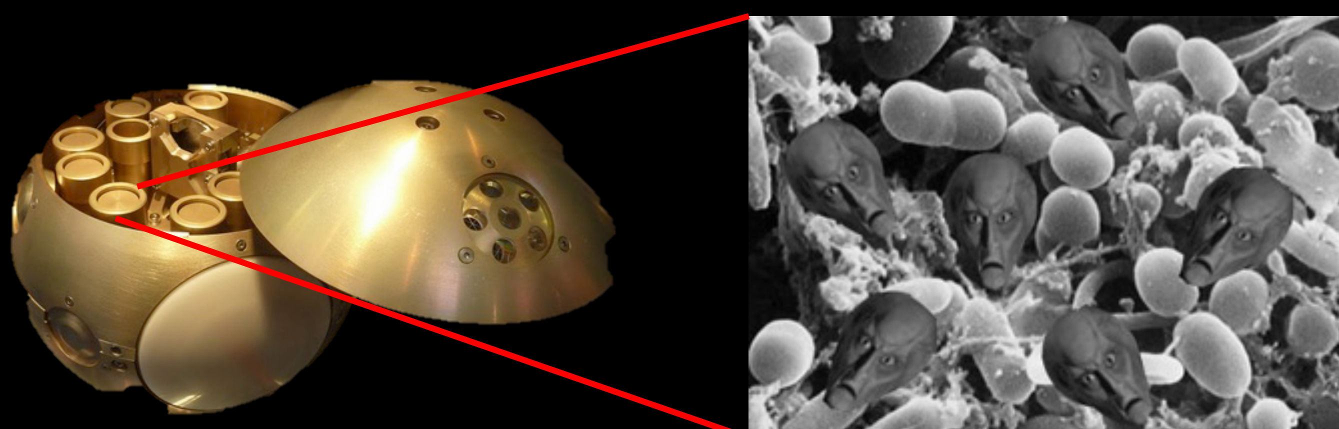


Salmonella typhimurium (Food poisoning)- More virulent after growth in space!

Have ecological impacts!

What is Planetary Protection?

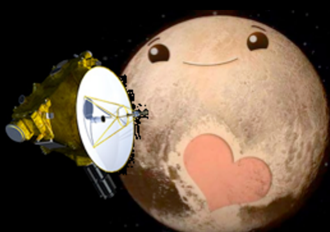
- Preserve planetary conditions for future biological and organic constituent exploration
  - *Prevent forward contamination*
- To protect Earth and its biosphere from potential extraterrestrial sources of contamination
  - *Prevent backward contamination*



How is Planetary Protection applied to missions?

Depending on where you are going…

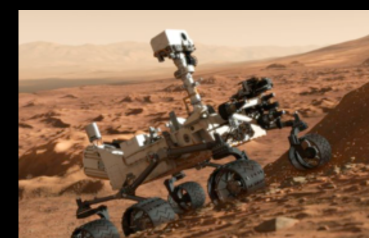
and what you are doing…



Fly-by

Orbiter

Lander



Rover

the Mission is assigned a Planetary Protection mission category which comes with cleanliness and documentation requirements.

Cleaning hardware is easy, but keeping it clean…

Humans contain 3×10^{13} human cells and 3.9×10^{13} microbes!

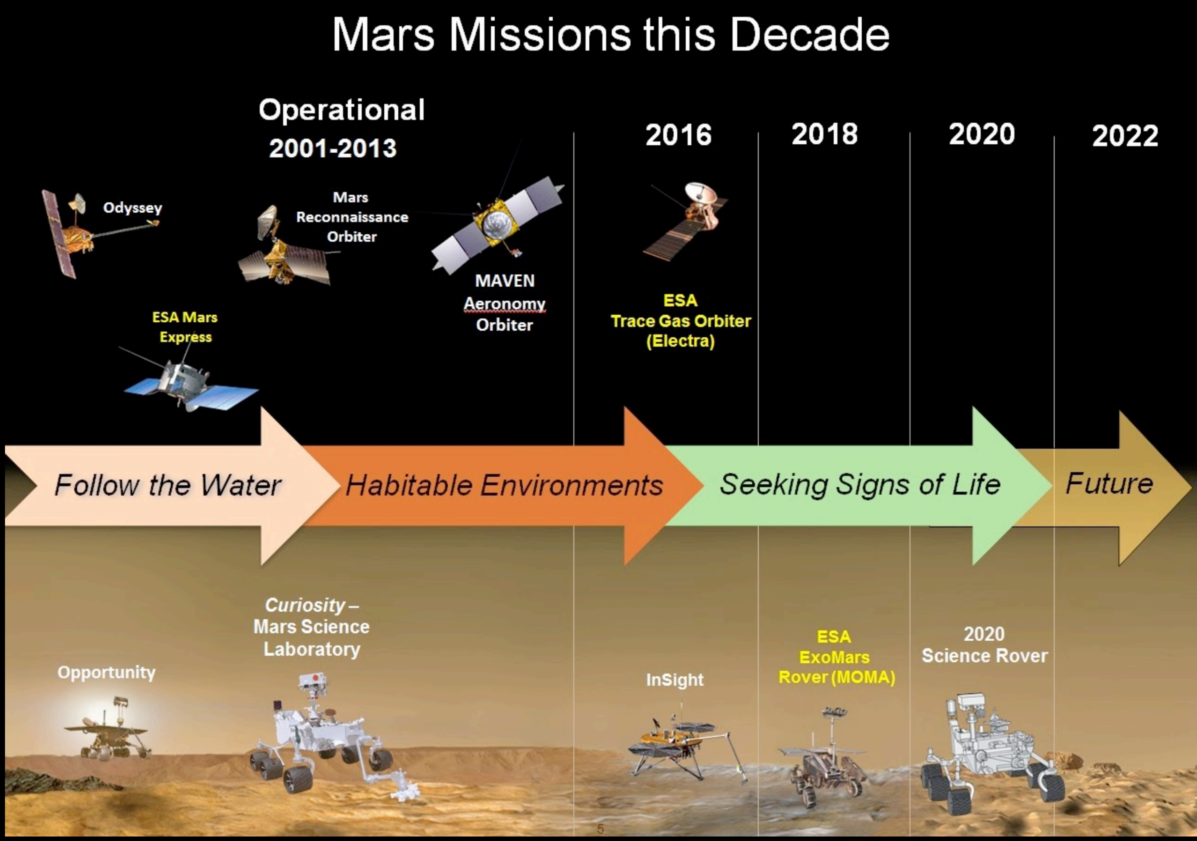
Autoclave

Viking Casserole: 120°C for 40 hours

NASA Standard Spore Assay – assesses the cleanliness of hardware to meet biological burden requirements. Selects for viable, cultivable, aerobic, heterotrophic spores, 32°C, 3 days

Planetary Protection has been implemented across several missions

Mission	Year Launched	Cateorization
Viking	1975	IV
Galileo	1989	II
Mars Global Surveyer	1996	III
Mars Pathfinder	1996	IV
Cassini	1997	II
Deep Space 1	1998	III
Mars Climate Orbiter	1998	
Mars Polar Lander	1998	IVa
Deep Space 2	1999	IVa
Stardust	1999	II/V
Mars Odyssey	2001	III
Mars Exploration Rover	2003	IVa
Rosetta	2004	II
Deep Impact	2005	II
MRO	2005	III
Venus Express	2005	I
Dawn	2007	III
Phoenix	2007	IVc
Kepler	2009	
Juno	2011	II
MSL	2011	IVc
InSight	2018	IVa
Mars 2020	2020	V Restricted Earth Return



… and will continue to be implemented across future missions as science goals and needs evolve.

Mission Overview

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LAUNCH

- Atlas V 541 vehicle
- Launch Readiness Date: July 2020
- Launch window: July/August 2020

CRUISE/APPROACH

- ~7 month cruise
- Arrive Feb 2021

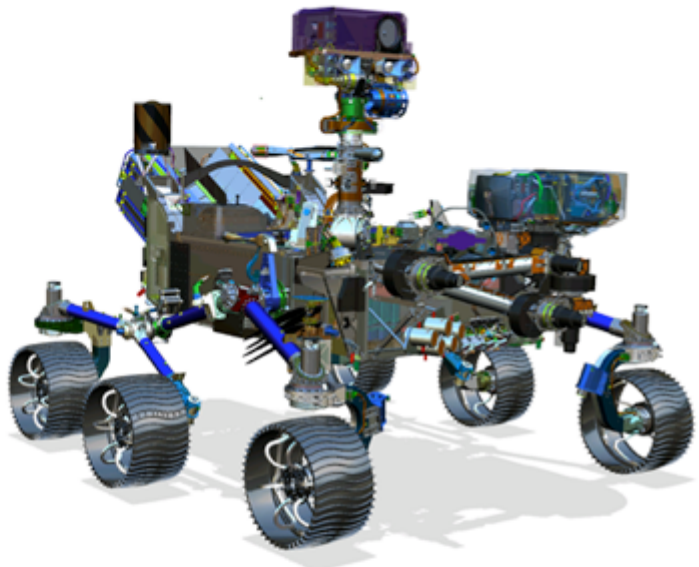
ENTRY, DESCENT & LANDING

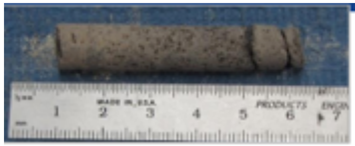
- MSL EDL system (+ Range Trigger and Terrain Relative Navigation): guided entry and powered descent/Sky Crane
- 16 x 14 km landing ellipse (range trigger baselined)
- Access to landing sites ±30° latitude, ≤ -0.5 km elevation
- Curiosity-class Rover

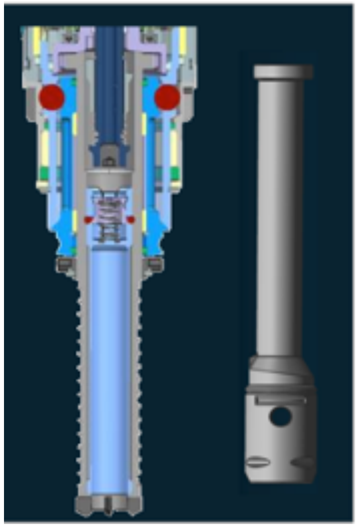
SURFACE MISSION

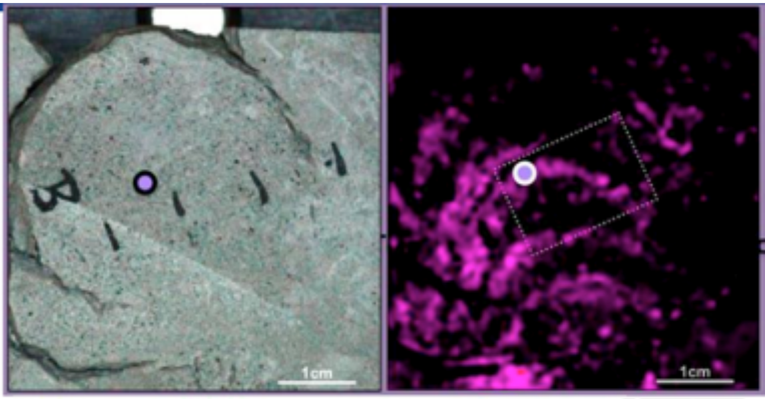
- 20 km traverse distance capability
- Enhanced surface productivity
- Qualified to 1.5 Martian year lifetime
- Seeking signs of past life
- Returnable cache of samples
- Prepare for human exploration of Mars

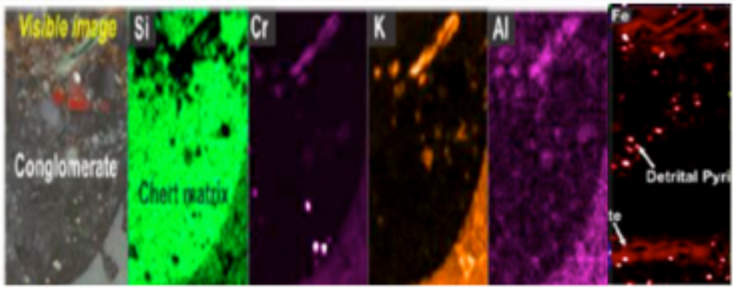
Mars 2020 Overview











Science

- *Assess past habitability of an astrobiologically relevant ancient environment on Mars*
- *Assess biosignature preservation potential with the environment and search for biosignatures*
- *Assemble cached samples for possible future return to Earth*

Technology

- *Advance technologies with applications to future human and robotic explorations objectives*

External Rover Configuration

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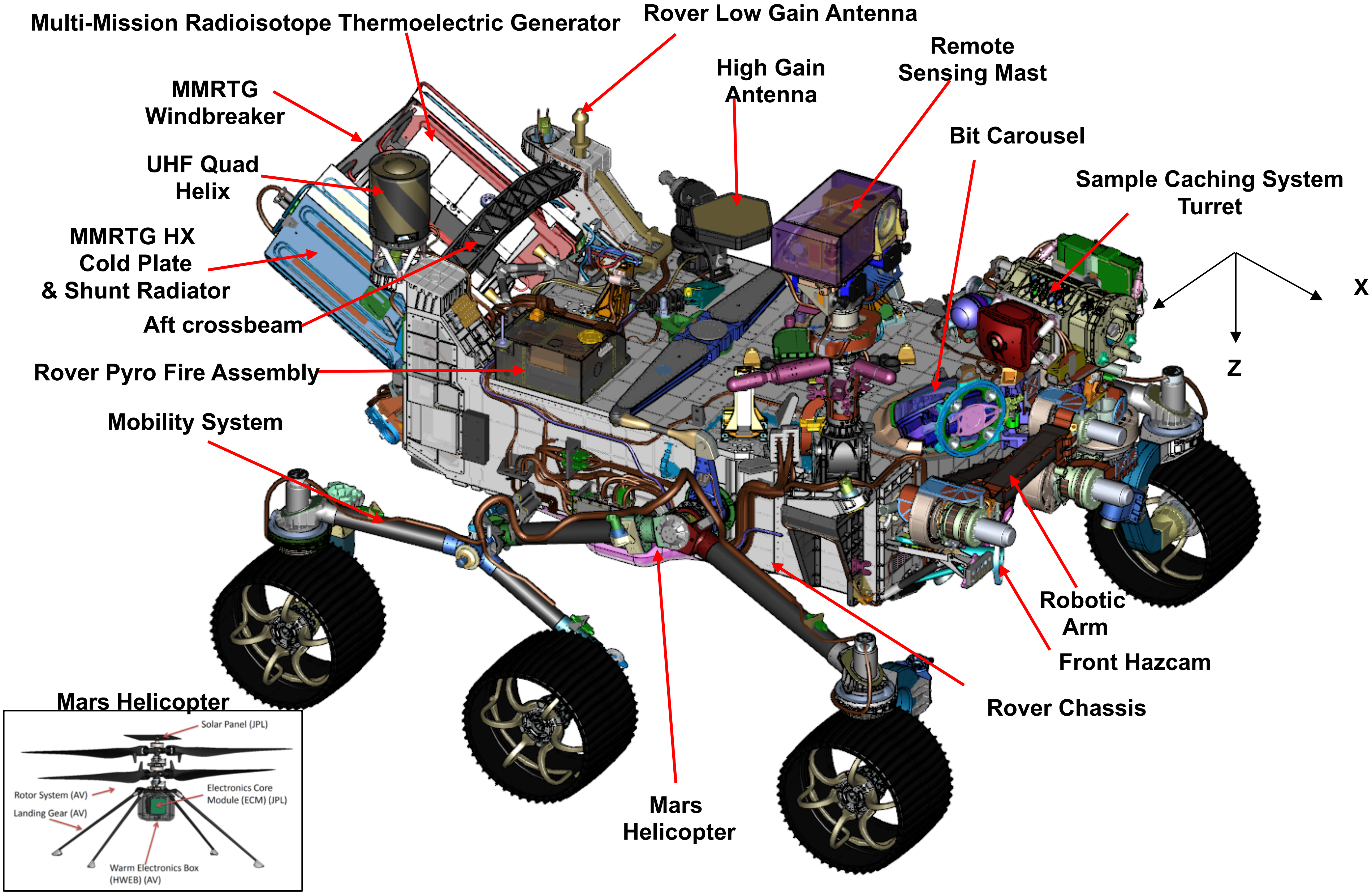

Sampling and Caching System Hardware Overview

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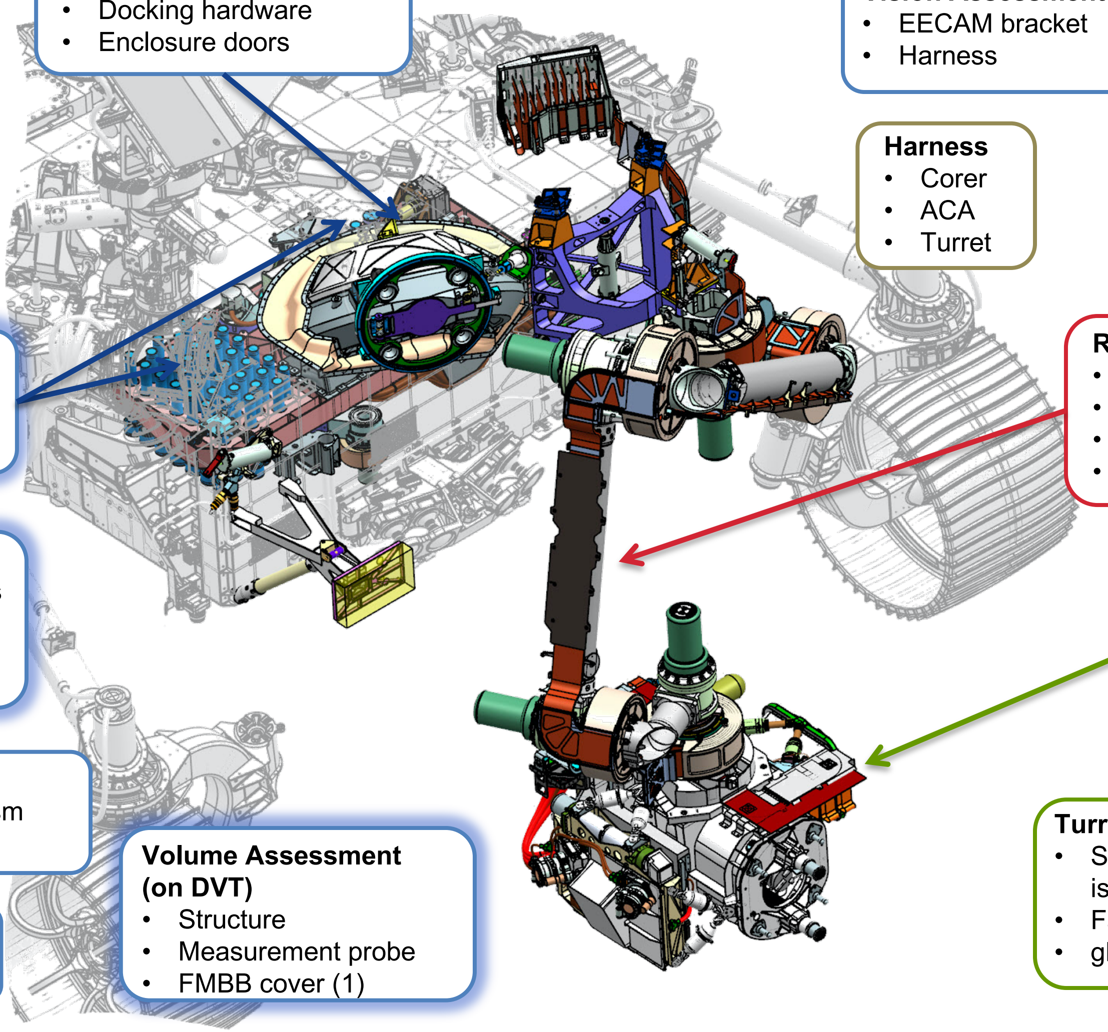

Sample Handling Assembly (SHA)
- Robotic arm
- Flex harness
- End effector

Bit Carousel
- Carousel / structure
- Docking hardware
- Enclosure doors

Vision Assessment
- EECAM bracket
- Harness

Harness
- Corer
- ACA
- Turret

Turret / Corer

Adaptive Cache Assembly (ACA)

Robotic Arm / Turret

Harness

SFM Hardware

Sample Tube Storage
- Storage structure
- Sample tubes (43)
- FMBB gloves (43)

Robotic arm
- Launch lock
- Flex harness
- 6DOF force/torque sensor
- Final actuator stages

Seal Dispenser (on DVT)
- Dispensing mechanisms
- Hermetic seals (43)
- FMBB covers (12)

Coring Drill
- Drill body
- Feed / turret structure
- Stabilizers
- Bits

Sealing / Drop-off
- Tube gripper mechanism
- Sealing mechanism

Volume Assessment (on DVT)
- Structure
- Measurement probe
- FMBB cover (1)

Turret
- SHERLOC VIBRATION isolators
- Facility contact sensor
- gDRT

FMBB Parking Lot
- Secondary structure

M2020 E2E Biological Contamination Performance

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Continuous models as function of time:

F(t)

- Ad(t) = particle adhesion vs. time
- $dm_{microbe}/dt$ = rate of growing/dying microbes (from Earth inventory)

Cruise
- Shock/vibration (e.g., thruster firings)
- Line-of-sight transport / elastic collisions

Repressurization
- Repressurization rate
- Induced velocity field

EDL Cleaning (Wind)
- Velocity field on rover

Launch
- Depressurization rate
- Induced velocity field

Discrete Events

Initial Contamination Level

EDL Cleaning (MLEs)

UV Kill

Commissioning
- Percussive cleaning

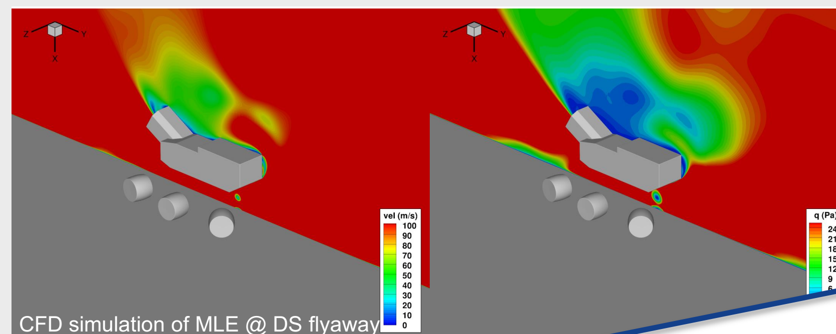
Operations

Each individual phase consists of:

Microbial Particle Transport Model

Particle Liberation → Particle Transport → Particle Deposition

Environments Model & Hardware State



BIOLOGICAL PARTICLE RESUSPENSION AND TRANSPORT

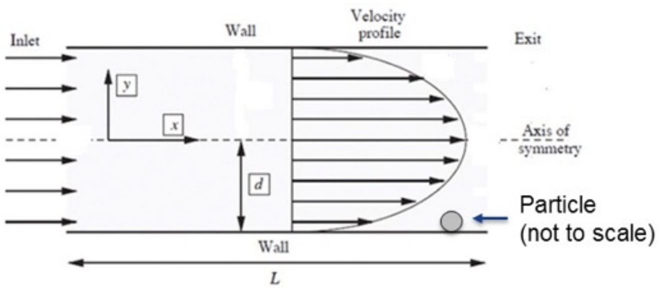
Particle Resuspension & Transport Physics
Ioannis (Yiangos) Mikellides
Nicole Chen
Stephen Liao
Evan Droz
Mark Anderson

Biology
Moogega Stricker
Fei Chen
Wayne Schubert
Ganesh Babu Malli Mohan

Ioannis Mikellides, et al. Theoretical Modelling and Experiments in Particle Resuspension and Transport for the Assessment of Terrestrial-Borne Biological Contamination of the Samples on the Mars 2020 Mission. 14th International Symposium on Materials in the Space Environment. Oct 1-5, 2018. In review.

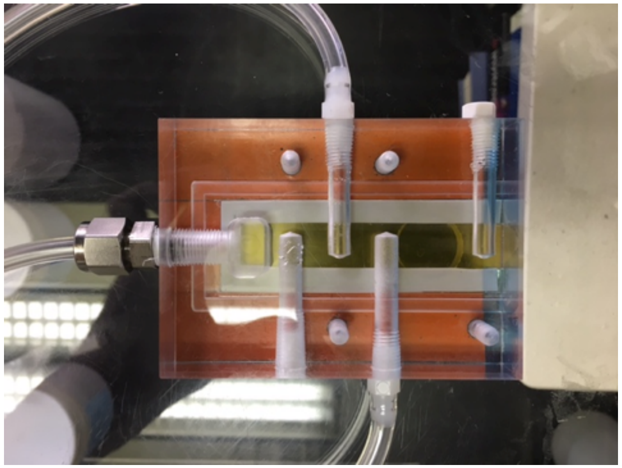
Controlled Experiments of Flow-induced Particle Resuspension

- Laminar flow device used successfully at Caltech by Prof. Flagan and students, and at JPL for all initial glass on glass tests.
- Identical test cell made of transparent thermoplastic (acrylic) developed and used for all particle (glass & dust) on s/c surfaces.
- Main advantage of the device: Establishes fully-developed laminar flow in the channel for which shear stress at the wall is well known by theory; no direct measurement of u* is needed.
- Device setup recently enclosed in purge box to control relative humidity (RH<20%); allowed for the completion of 100+ experiments during ~10 weeks



Metallic Flow Device (courtesy Prof. Flagan, Caltech) – used for glass-on-glass resuspension tests

Acrylic (transparent) Flow Device (JPL) – used for dust on s/c surfaces resuspension tests



Biological particle resuspension

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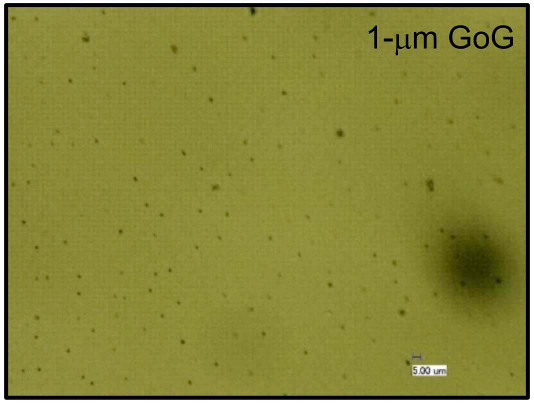
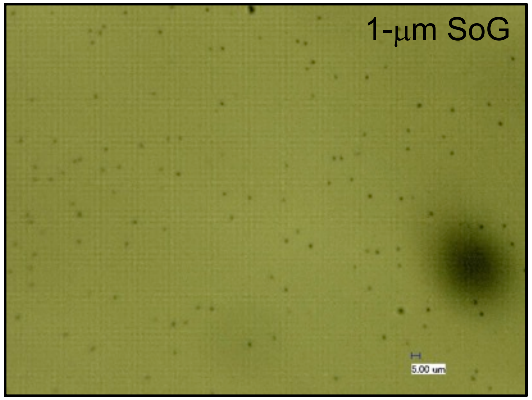
- Experiments were performed to compare the lateral force needed to release biological and non-biological particles of the same size.
- Flow-induced resuspension using laminar flow test cell
  - Substrate material: Glass
  - Particles of diameter ~1 μm
    - Biological: BSN
    - Non-biological: glass sphere
  - Approach: apply flow induced (lateral) forces after taking into account mission exposure conditions, and assess resuspension at various phases.

Biological particle resuspension
Flow-induced Resuspension Tests. Description

- **Main objective**: Use laminar flow test system to apply a shear (lateral) force on biological and non-biological particles and determine their particle removal fractions (PRF). By comparison, determine which of the two particle types has a higher removal fraction at fixed shear force.
- **Experiment**
  - Expose particle-deposited coupons to ATLO, launch, and cruise conditions and measure PRFs at the end of all exposures.
  - Conditions
    - Residence time: 10 days
    - Expose to low humidity
    - Undergo temperature cycling (0 days to simulate pre-landing, 3 days near real-time Mars temperature cycle condition)
  - Substrate material: Glass
  - Particles of diameter ~1 μm
    - Biological Particle: *Bacillus atrophaeus 9372 endospores*
    - Non-biological: glass sphere





Biological particle resuspension
Experimental Flow Emulates Mission Exposure Conditions

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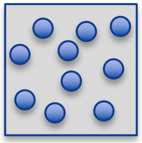
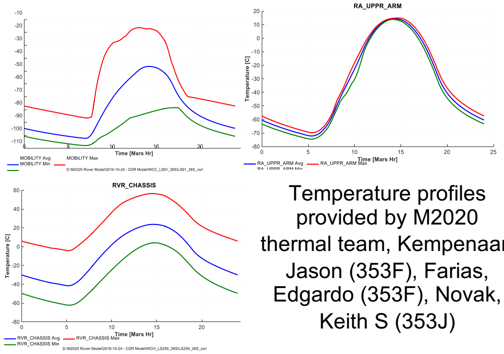


Microbe deposition

Lyophilization (-80C for 4 hrs,150 mtorr for 13 hours)

Transport to thermal chamber under vacuum

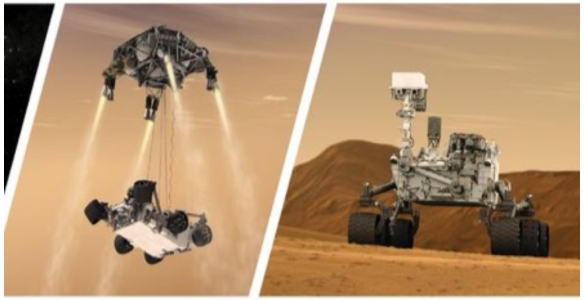


Temperature profiles provided by M2020 thermal team, Kempenaar, Jason (353F), Farias, Edgardo (353F), Novak, Keith S (353J)

Thermal Cycling

ATLO

LAUNCH CRUISE/APPROACH



ENTRY, DESCENT & LANDING SURFACE MISSION

= Wind Resuspension Exposure

Pre-lyophpolization, **Pre**-exposure to thermal-cycling (9/19/2017)

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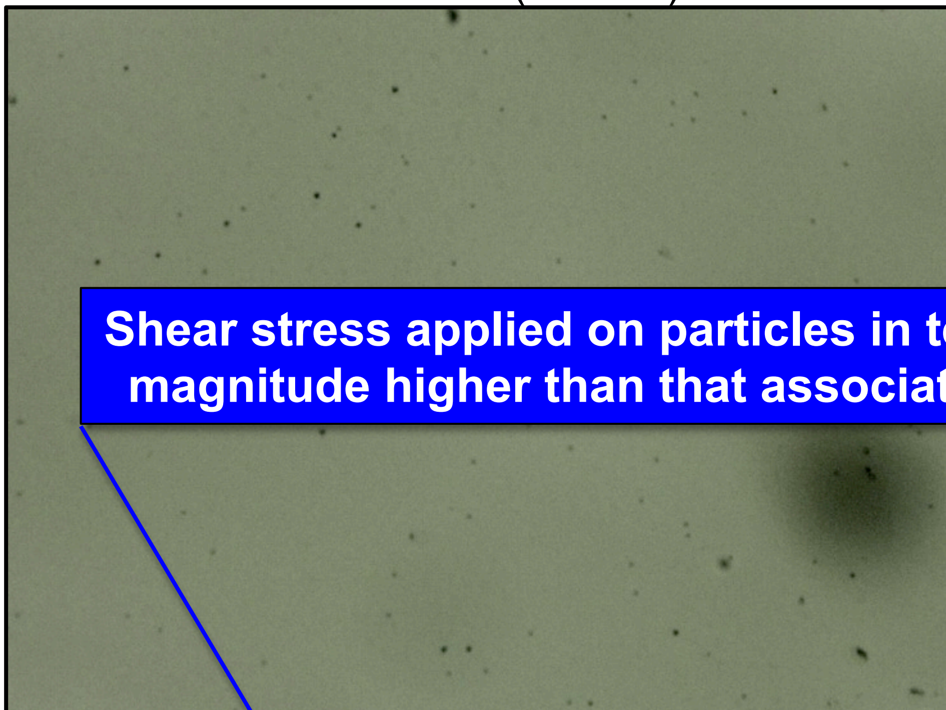
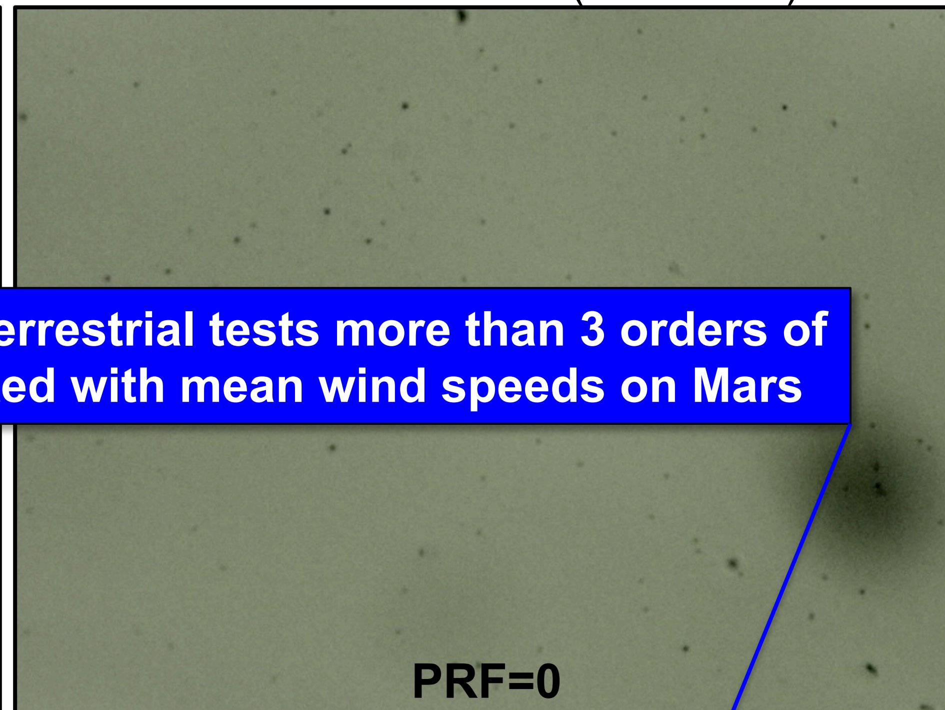
Glass particles on Glass substrate (GoG)

Before Flow (0 L/min)



After Maximum Flow (9.92 L/min)



Shear stress applied on particles in terrestrial tests more than 3 orders of magnitude higher than that associated with mean wind speeds on Mars

Condition	Earth	Mars	
ρ (kg/m^3)	1.2	0.022	
u_∞ (m/s)	34.5 (Q=10 L/min)	4.7 (mean wind)	15.3 (99-% wind)
τ_w (Pa)=ρu_*^2	10.1 (Channel LBL)	0.007 (Plate TBL)	0.06 (Plate TBL)

TBL=Turbulent boundary layer
LBL=Laminar boundary layer

Post-lyopholization, **Post**-exposure to thermal-cycling (9/25/2017)

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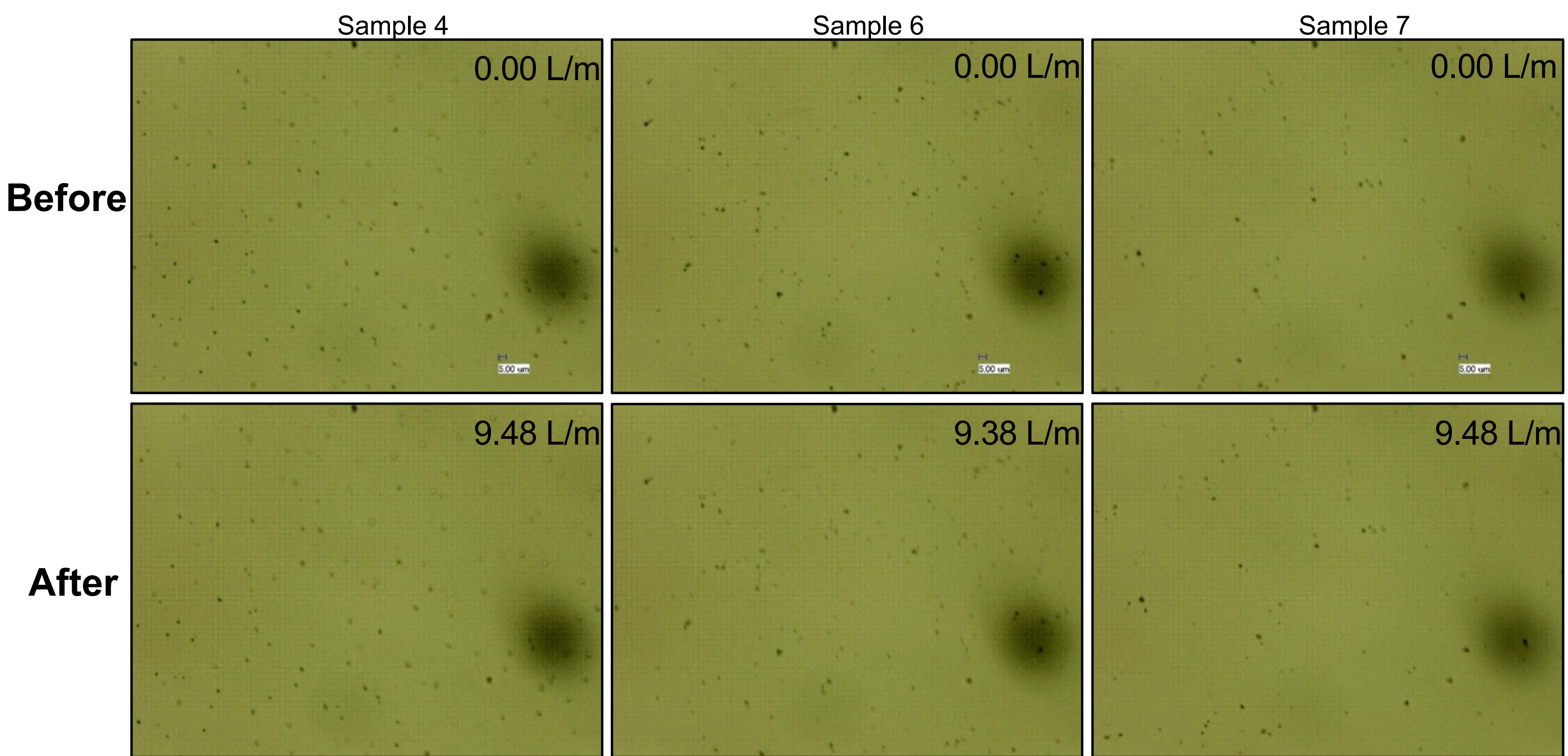
Test	Conditions	PRF at max flow rate	Particle surface density (#/cm^2)
GoG - Sample 1	**Post**-L, **Pre**-T	0	156250
GoG - Sample 2	**Post**-L, **Pre**-T	0	279948
GoG - Sample 3	**Post**-L, **Pre**-T	0	141927
GoG - Sample 4	**Post**-L, **Post**-T	0	177083
GoG - Sample 6	**Post**-L, **Post**-T	0	164063
GoG - Sample 7	**Post**-L, **Post**-T	0	121094
GoG - Sample 9	**Pre**-L, **Pre**-T	0	174479
GoG - Sample 10	**Pre**-L, **Pre**-T	0	119792
GoG - Sample 11	**Pre**-L, **Pre**-T	0	143229

Test	Conditions	PRF at max flow rate	Particle surface density (#/cm^2)
SoG - Sample 13	**Post**-L, **Pre**-T	0	101563
SoG - Sample 14	**Post**-L, **Pre**-T	0	84635
SoG - Sample 15	**Post**-L, **Pre**-T	0	195313
SoG - Sample 16	**Post**-L, **Post**-T	0	214844
SoG - Sample 18	**Post**-L, **Post**-T	0	151042
SoG - Sample 19	**Post**-L, **Post**-T	0	203125
SoG - Sample 21	**Pre**-L, **Pre**-T	0	158854
SoG - Sample 22	**Pre**-L, **Pre**-T	0	119792
SoG - Sample 23	**Pre**-L, **Pre**-T	N/A	N/A



Summary Remarks

- Biological contamination due to resuspension of biological particles
  - Environment during flow tests emulated mission phase conditions
  - Flow-induced resuspension tests performed at JPL comparing 1-μm spores and glass spheres on glass substrates. Are resuspension characteristics of biological particles different than those of non-biological particles of comparable size?
    - Flow tests show no removal of 1-μm particles under an applied shear stress that is 2-3 orders of magnitude greater than that expected on Mars during nominal wind conditions.
    - Result independent of mission phase environment.

M2020 E2E Biological Contamination Performance

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Continuous models as function of time: F(t)

- $Ad(t)$ = particle adhesion vs. time
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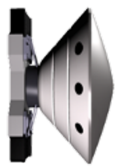
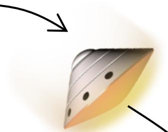
Cruise
- Shock/vibration (e.g., thruster firings)
- Line-of-sight transport / elastic collisions





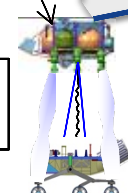
Repressurization
- Repressurization rate
- Induced velocity field

EDL Cleaning (Wind)
- Velocity field on rover

Launch
- Depressurization rate
- Induced velocity field

Discrete Events

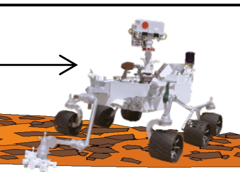
Initial Contamination Level



EDL Cleaning (MLEs)

UV Kill

Commissioning
- Percussive cleaning



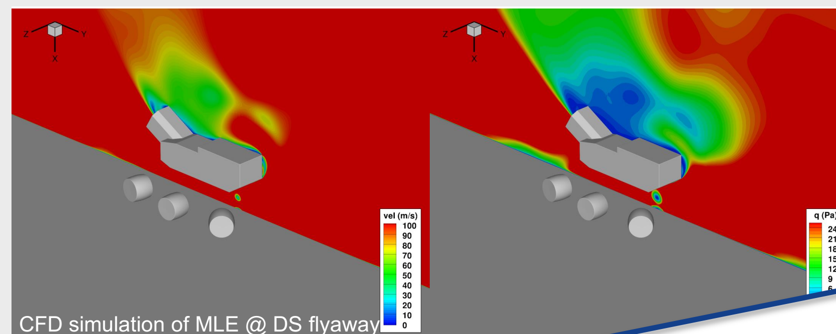
Operations

Each individual phase consists of:

Microbial Particle Transport Model

Particle Liberation → Particle Transport → Particle Deposition

Environments Model & Hardware State

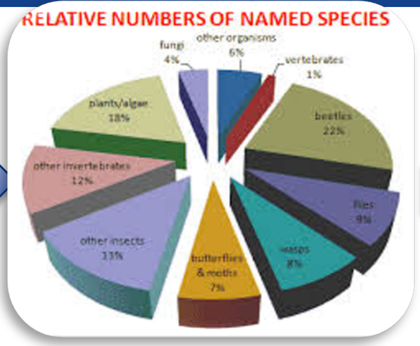


CFD simulation of MLE @ DS flyaway

Microbial Inventory Cataloging

Grow

Identify



Arch

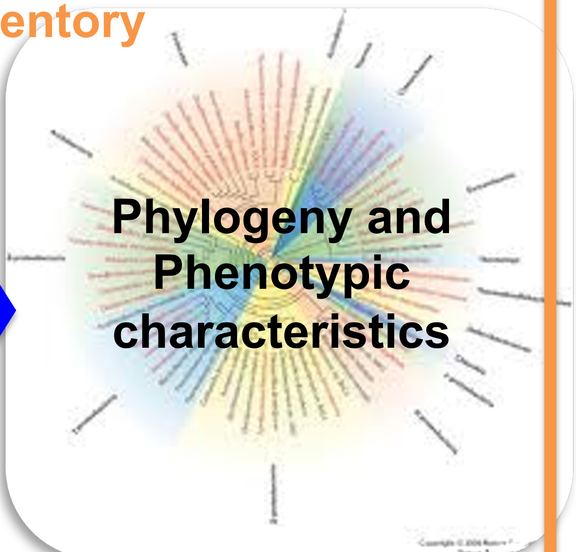
Traditional culturing takes >7 days to complete; Coverage is only <1 to 10%

Molecular method takes <3 days to complete and yield ~>90-fold diversity

Analysis Pathway for Genetic Inventory

Archive

High Throughput sequencing



MEP Historical work: MSL Genetic Inventory Approach

Jet Propulsion Laboratory
California Institute of Technology

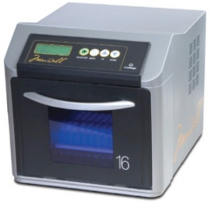
Sampling
- Wash first prior to sampling and use as control
- Wash BisKit 3 times in total and pool

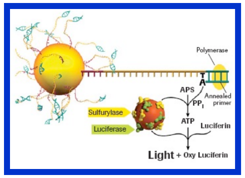
Sample concentration
- Use amicon filters and concentrate

Sample extraction
- Use Maxwell and split DNA into 3 portions

Analysis
- PhyloChip
- Next-gen sequencing
- Archive 2 portions



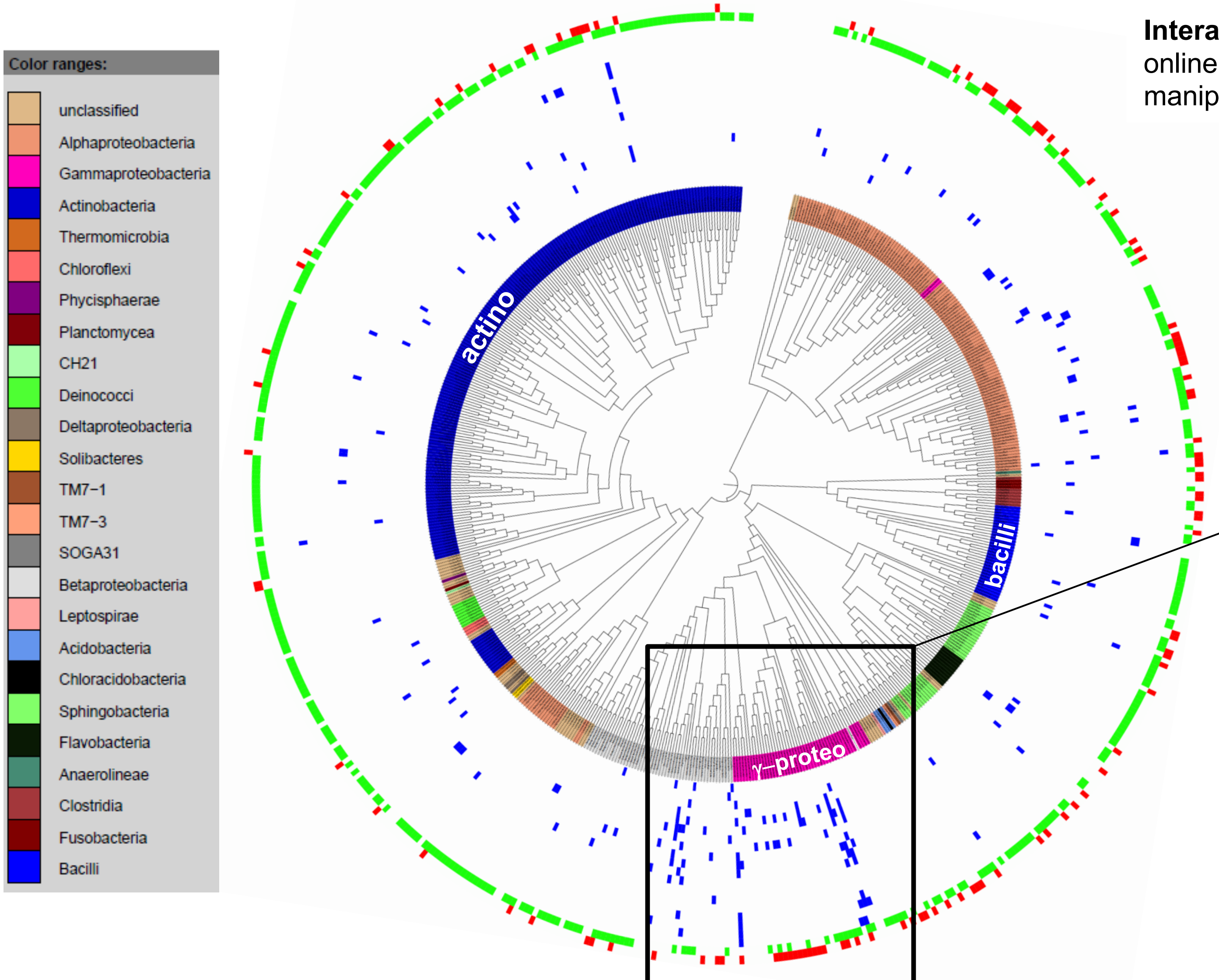





Standardized protocols and procedures based on assembly facility samples were implemented on spacecraft hardware samples

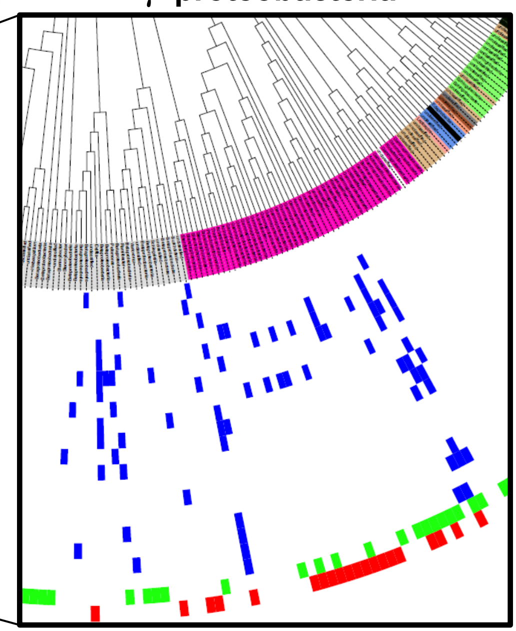
iTOL tree (Bacterial pyrosequences >350-bp)

Interactive Tree Of Life (iTOL) is an online tool for the display and manipulation of phylogenetic trees.



JPL-SAF cleanroom floor
JPL-SAF GSE
MSL spacecraft surfaces

γ–proteobacteria



The presence of OTU representative of actinobacteria, deinococci, acidobacteria, firmicutes, and proteobacteria on spacecraft surfaces suggests that certain bacterial lineages persist even following rigorous quality control and cleaning practices.

MT LaDuc, et al. Pyrosequencing-derived bacterial, archaeal, and fungal diversity of spacecraft hardware destined for Mars. Appl Environ Microbiol. Aug 2012;78(16):5912-22.

Identification of novel bacterial isolates from spacecraft and associated surfaces

International Journal of Systematic and Evolutionary Microbiology (2013), **63**, 2463–2471 **DOI** 10.1099/ijs.0.047134-0

Description of *Tersicoccus phoenicis* gen. nov., sp. nov. isolated from spacecraft assembly clean room environments

Parag Vaishampayan,[1] Christine Moissl-Eichinger,[2] Rüdiger Pukall,[3] Peter Schumann,[3] Cathrin Spröer,[3] Angela Augustus,[4] Anne Hayden Roberts,[4] Greg Namba,[4] Jessica Cisneros,[4] Tina Salmassi[4] and Kasthuri Venkateswaran[1]

International Journal of Systematic and Evolutionary Microbiology (2010), **60**, 1031–1037 **DOI** 10.1099/ijs.0.008979-0

Bacillus horneckiae sp. nov., isolated from a spacecraft-assembly clean room

Parag Vaishampayan,[1] Alexander Probst,[1]† Srinivasan Krishnamurthi,[2] Sudeshna Ghosh,[1] Shariff Osman,[1]‡ Alasdair McDowall,[3] Arunachalam Ruckmani,[2] Shanmugam Mayilraj[2] and Kasthuri Venkateswaran[1]

…, but tomorrow's needs require an unending search for new technologies

- Cross-disciplinary synergies
- High throughput sample processing
  - Sourced from Spacecraft, Spacecraft Assembly Facility (surface and air), and Ground Support Equipment.
  - Low biomass samples
- Long-term storage of:
  - Bioinformatics data
  - DNA
  - Microbial isolates from NASA Standard Assay
- **DNA-based techniques are evolving over time and so long-term storage of samples is important for processing using the technology of tomorrow**

MSL samples	**Total Processed**
Wipes	1206
Swabs	3188

Summary

- Lyophilization has been used and continues to be an essential tool that is leveraged in the space industry.
- Freeze Drying is advantageous to solve several problems:
  1. Preservation of products with a limited shelf life
     - Food
     - Reagents
  2. Transportation and storage ease
     - Food
     - Reagents
     - Waste products
  3. Relevant simulation conditions for space research
     - Developing a fundamental understanding of the lyophilization process
     - Simulating microbial physical state that is exposed in space, including the cruise phase of an outbound spacecraft.

Jet Propulsion Laboratory
California Institute of Technology